Supporting Information

Morton *et al*. 10.1073/pnas.0804673105

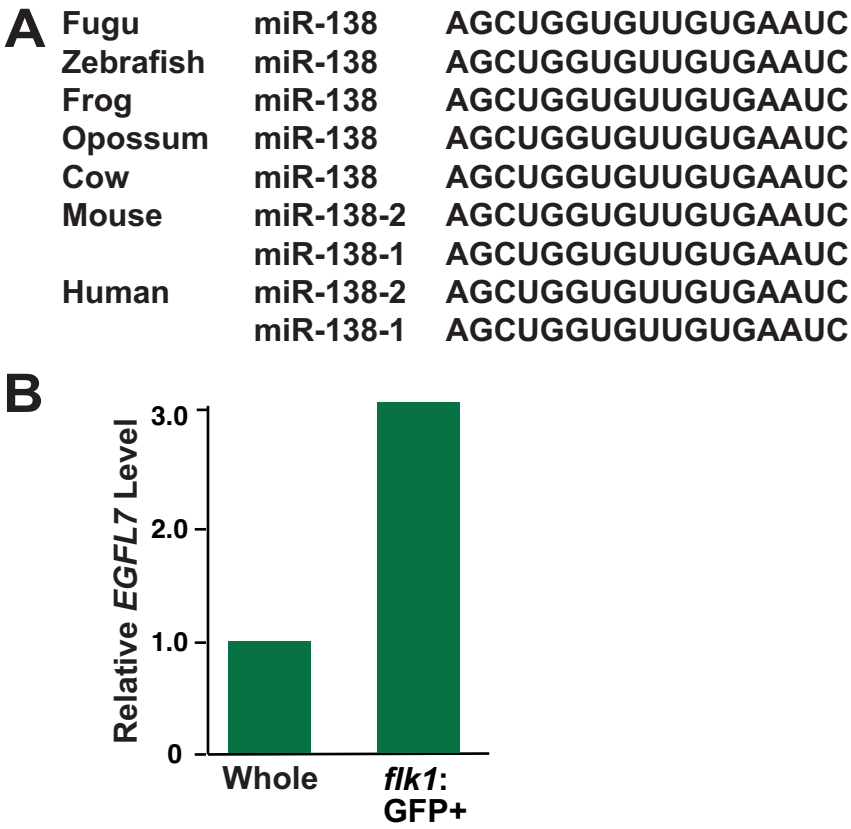


Fig. S1. (*A*) Alignment of mature miR-138 sequence from multiple species. A single copy of the gene is present in most species shown, whereas 2 genes encoding miR-138 are found in others, including mouse and human. (*B*) qRT-PCR analysis of expression of the endothelial-specific gene *EGFL7* in *flk1*:GPF^{+} cells isolated by flow cytometry demonstrates enrichment in the GPF^{+} population compared with whole embryo.

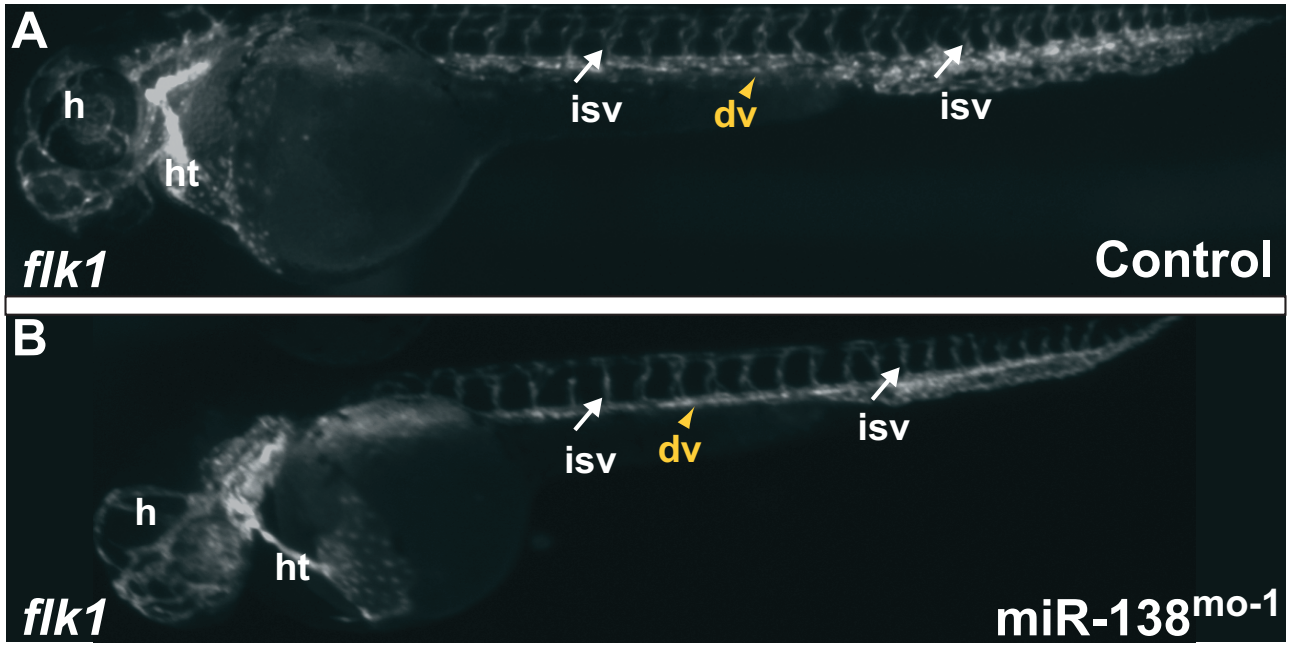


Fig. S2. (*A* and *B*) *Tg(flk1:EGFP)*^{s843} embryos, where GFP expression at 48 hpf marks endocardial cells and endothelial cells in blood vessels. Blood vessels between the somites and within the head appear similar in control (*A*) and miR-138-morpholino injected fish (*B*). (dv, dorsal vessels; h, head; ht, heart; isv, intersomitic vessels.)

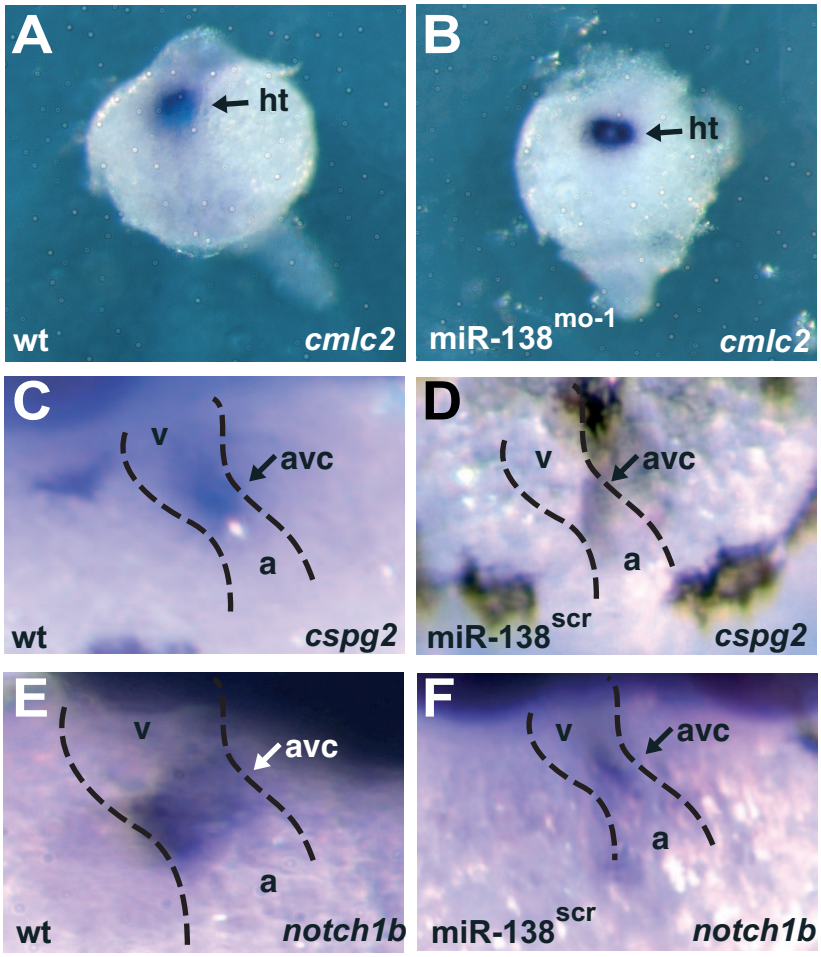


Fig. S3. (*A* and *B*) Expression of *cmlc2* in dorsal views at 24 hpf as determined by in situ hybridization (purple) in whole-mount embryos indicates that cardiac progenitors are similarly specified in WT control (*A*) and 138-morpholino-injected fish (*B*). (*C–F*) In situ hybridization for *cspg2* (*C* and *D*) and *notch1b* (*E* and *F*) in wild-type (*C* and *E*) and miR-138^{scr} fish (*D* and *F*) demonstrate atrioventricular-specific expression at 48 hpf. a, atrium; ht, heart progenitors; v, ventricle.

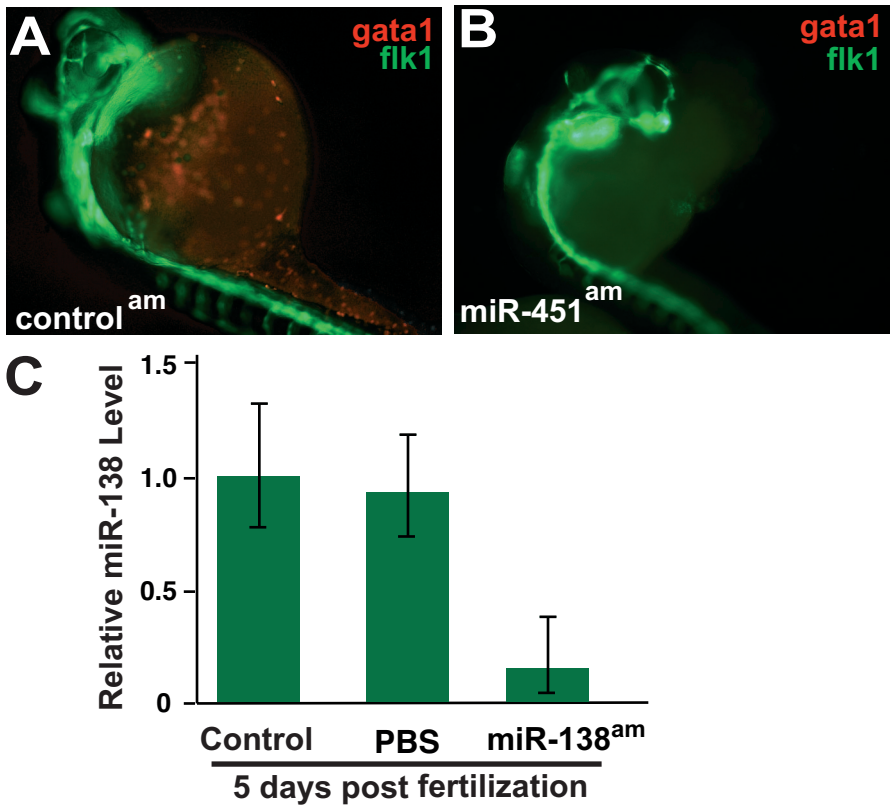


Fig. S4. (*A* and *B*) *Tg*(*flk1:EGFP*^{s843}; *gata1:dsRed*)^{s843} embryos at 32 hpf, where RFP expression marks red blood cells (RBCs) and GFP expression marks endothelial cells. Fewer RBCs were seen in miR-451^{am} embryos (*B*) than in control antagomiR-treated embryos (*A*). (*C*) qRT-PCR analysis of 5-day postfertilization embryos treated with PBS or miR-138 antagomiR demonstrates continued knockdown of miR-138.

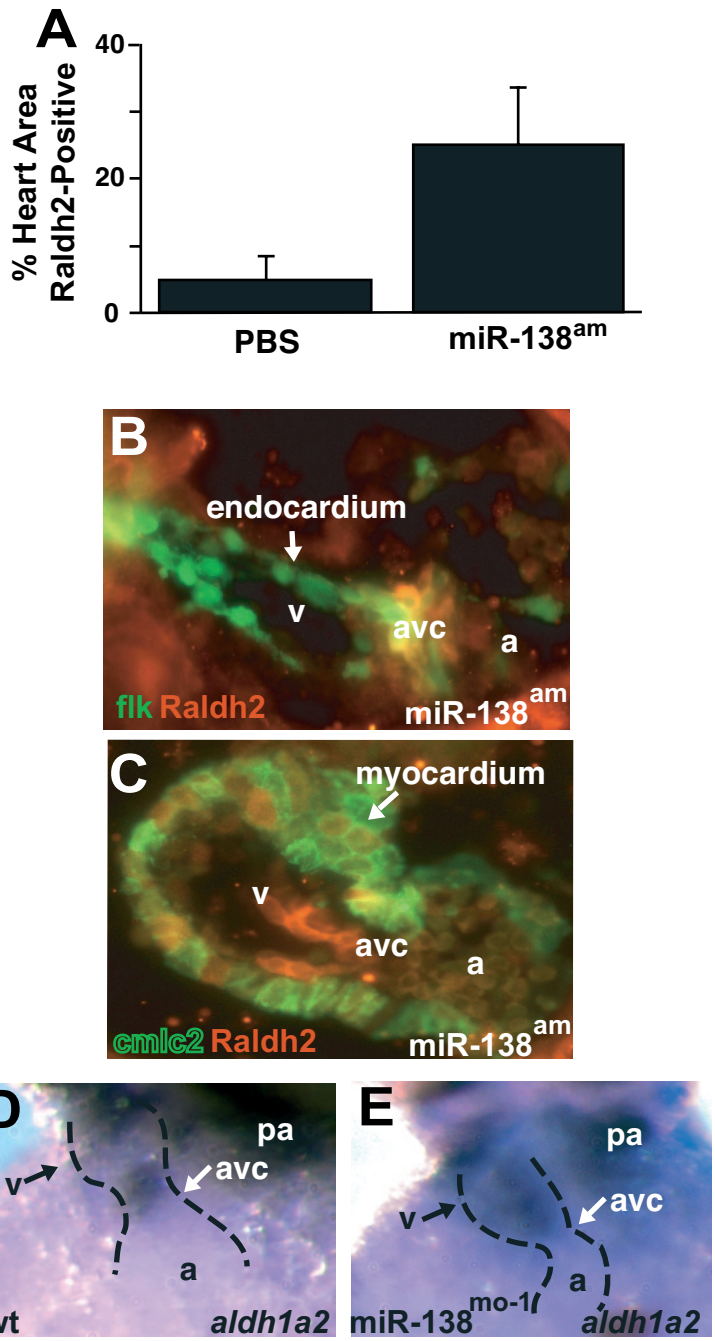


Fig. S5. (*A*) Quantification of Raldh2-positive area in multiple immunohistochemistry sections as represented in main text Fig. 4 *C* and *D*. Results shown are the average of 4 slides per group. (*B* and *C*) Immunohistochemistry for Raldh2 protein in miR-138^{am} fish demonstrates Raldh2 expression (red) overlapping with Tg(*flk1*-EGFP)^{s843} (*B*) in the atrioventricular canal (avc), and Raldh2 overlapping with Tg(*cmlc2*-ras-EGFP)^{s843} in the ventricle (v) (*C*). *cmlc2*-ras-EGFP expression is localized to the cell membrane of cardiomyocytes. (*D* and *E*) In situ hybridization for *aldh1a2* demonstrates AVC-restricted expression in control fish (*D*) but expanded ventricular expression in miR-138mo fish (*E*). wt, wild-type; pa, pharyngeal arch; a, atrium.

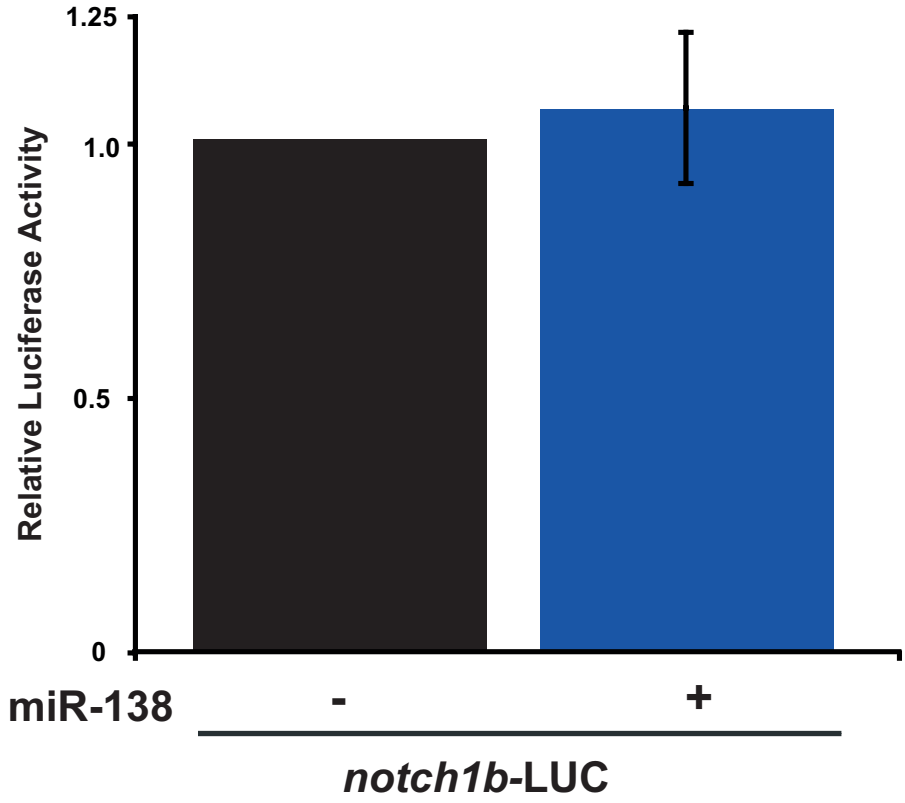
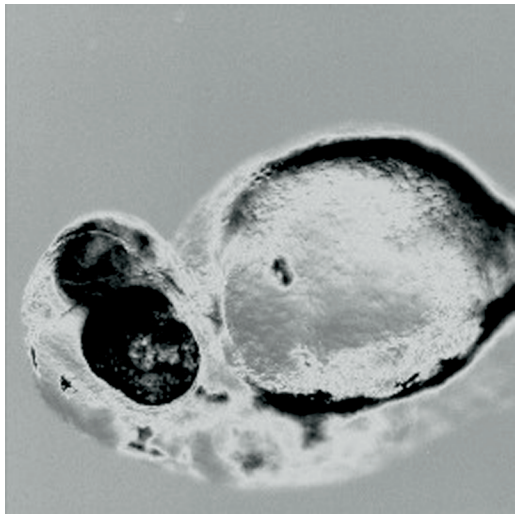
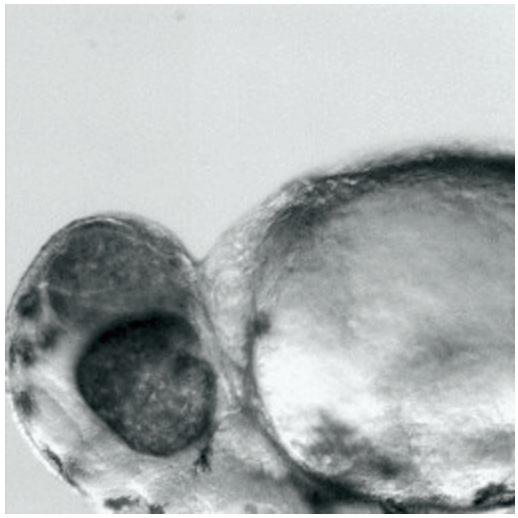


Fig. S6. Luciferase activity on introduction of *Danio rerio notch1b* 3′ UTR sequences downstream of a cytomegalovirus-driven luciferase reporter. Luciferase activity in Cos cells transfected with miR-138 is shown.



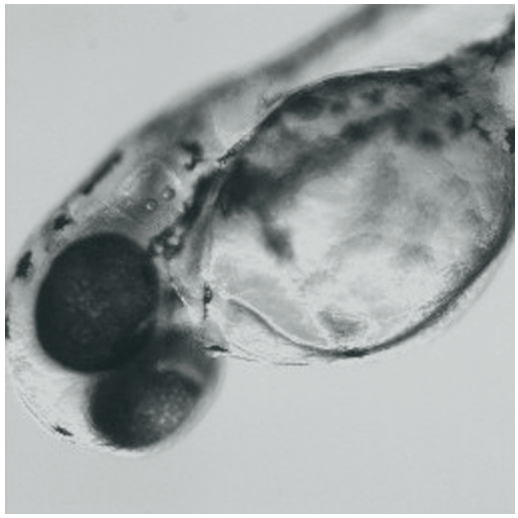
Movie S1. Video of fish embryos at 36 hpf shows that the cardiac function of mir-138^{scr} fish is similar to that of miR-138mo fish (Movie S2) with respect to heart rate, contractility, and circulation.

Movie S1



Movie S2. Video of fish embryos at 36 hpf shows the cardiac function of miR-138^{scr} fish (Movie S1) is similar to that of mir-138mo fish with respect to heart rate, contractility, and circulation.

Movie S2



Movie S3. Video of fish embryos at 48 hpf shows the cardiac function of miR-138^{scr} control embryos.

Movie S3

Movie S4. Video of fish embryos at 48 hpf shows that the cardiac function of miR-138mo embryos, which is notably different than in fish embryos at 36 hpf (Movies S1 and S2), with defects in cardiac looping and contractility not seen in miR-138^{scr} control embryos (Movie S3).

Movie S4